

Synchrotron Radiation in Biotechnology:

*Combining Physics, Biology,
Chemistry, and Mathematics to do
Science*

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Brookhaven Biology

The Plan for this Lecture

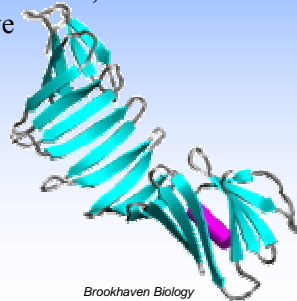
I'm going to take you through some of the adventures and successes scientists in the **Brookhaven Biology Department** have had in using Brookhaven's National Synchrotron Light Source to make discoveries that have medical or agricultural value.

For example....

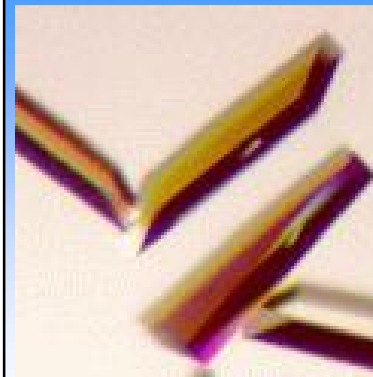
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Lyme disease is an increasing health concern on Long Island.

It affects more than 10,000 Americans each year, and it can be difficult to diagnose. Since it can cause severe complications if untreated, there is a real need for a safe and effective vaccine. This image shows the structure of **an important protein** that's located on the outer surface of the bacterium that causes **Lyme disease**. It's called **OspA**.



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To learn the structure of OspA, researchers in **Brookhaven's Biology Department** studied protein **crystals** like these using **x-ray diffraction**.

OspA was used in first-generation **Lyme disease vaccines** -- but differences among strains of bacteria can make a vaccination fail.

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New models of OspA's structure show exactly where **different strains of bacteria vary**.

Researchers can use this information to develop **second-generation vaccines** that will give better protection again Lyme disease.



C. Lawson, J. Dunn



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This study of the Lyme Disease Vaccine is just one example of the way **Brookhaven Biologists** are using **Biology, Chemistry, Physics, and Math** to answer questions that can be important to human health, to agriculture, and to fundamental science.

Let's look at how all these disciplines can fit together to provide this wealth of information....

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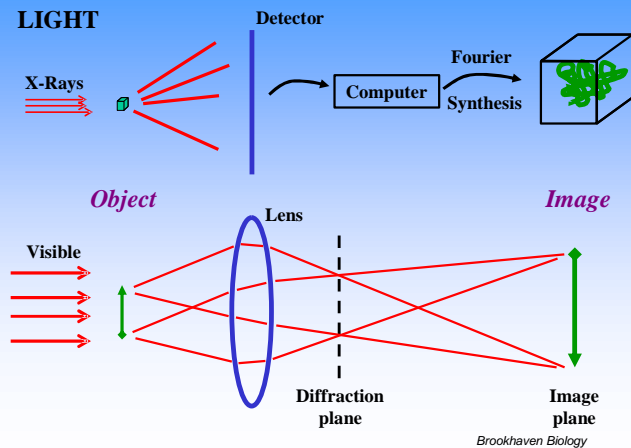
How does it work?

I've told you that we use **crystals** and **x-rays**. These allow us to "look" at large molecules like OspA.

You'll be surprised how much you already know about how we might do this.

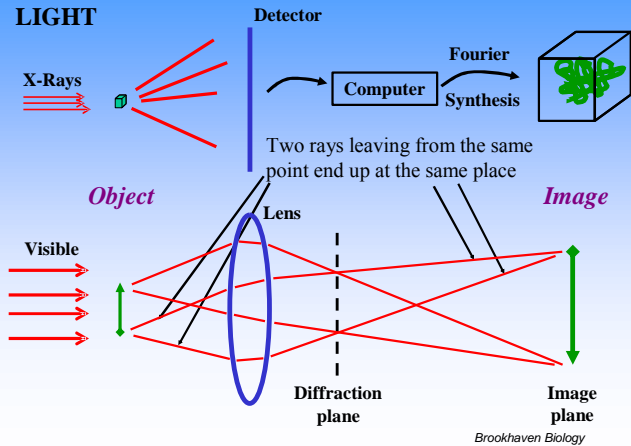
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Creation of a molecule's image from a crystal has similarities to creating an image with a lens

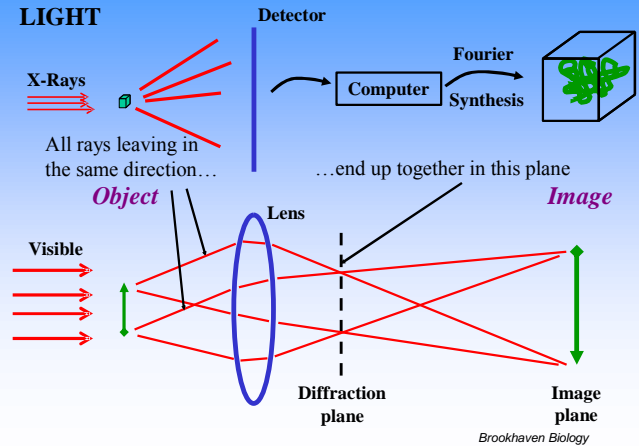


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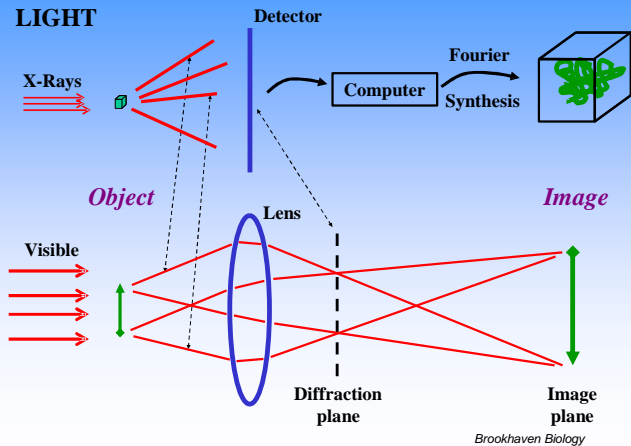
You already understand a little about how lenses work



Maybe you didn't know ...



We use a crystal to give us diffraction



Why do we use **x-rays**?

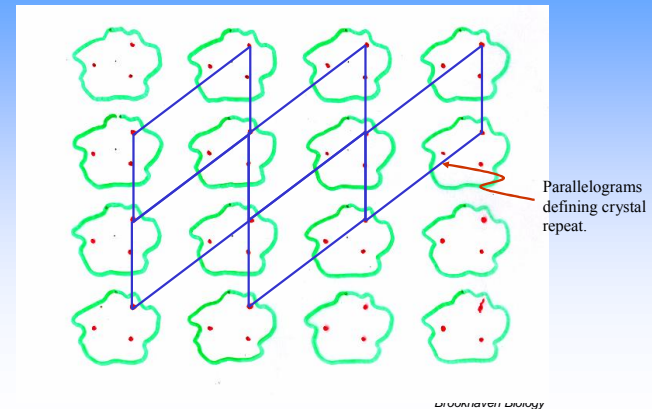
- The features we're trying to see are on the order of the **distance between atoms**: 10^{-10} meters.
- To "see" the atoms, we need to use light with a **wavelength** that is near to **this distance**.
- X-Rays (x-ray light) have a **suitable wavelength**.

What is a **crystal**?

- A crystal is a **periodic arrangement** of objects (molecules) repeating in two or three dimensions.
- The **repeating unit** is a parallelepiped (three-dimensional) or a parallelogram (two-dimensional).
- A crystal like that of OspA will be half a mm on a side and contain **10^{15} molecules**.

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Here's one choice of repeating unit in this crystal made of apple trees



Why do we use crystals when we'd like to see one molecule?

- We can't **focus** enough x-rays into a small enough volume to "see" a molecule. We use lots of molecules in a **crystal** to get a bigger target.
- Even if we could, the x-rays would **burn up** the molecule.
- Even if that would work, we **don't have a lens** for the x-rays.
- The crystal **amplifies the signal**, and gives us a way to get the **phase information** back.

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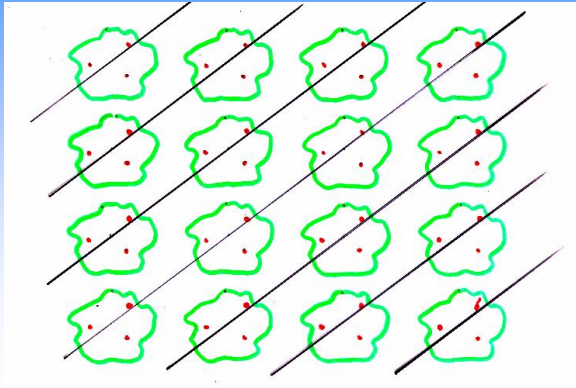
We can slice the crystal at **lattice points**: all planes pass through the same apple



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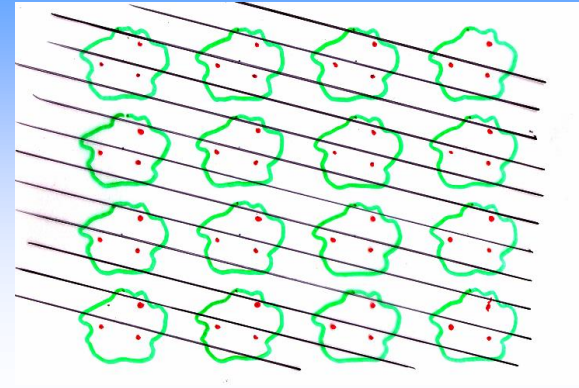
And at other angles. Notice:

- planes all pass by the same apple;
- the “stuff” between pairs of planes is always the same.



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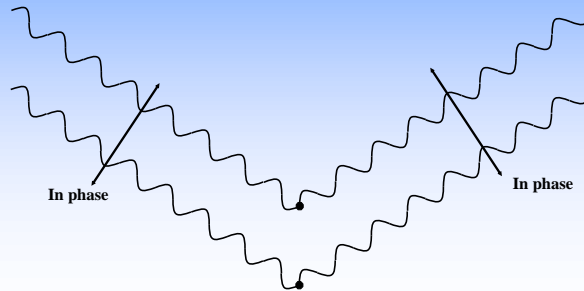
And one more time...



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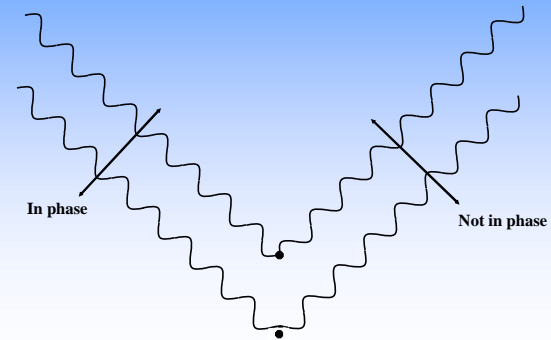
Diffraction

Let's do a thought experiment. Send a beam of x-rays at a pair of single atoms, suspended in space. If the angle is just right for the wavelength and distance between the atoms, the scattered x-rays will be in phase, and they will interfere constructively.



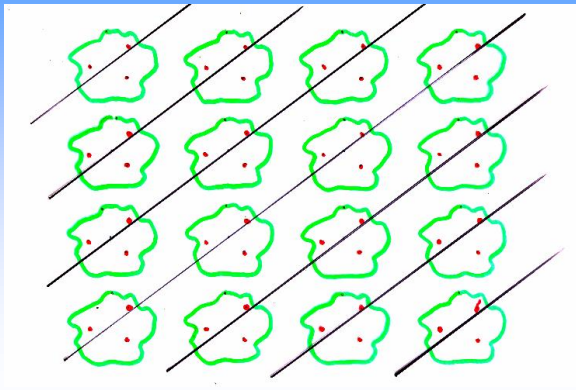
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On the other hand, if things are not right, they won't be in phase, and there will be no constructive interference, no diffraction.

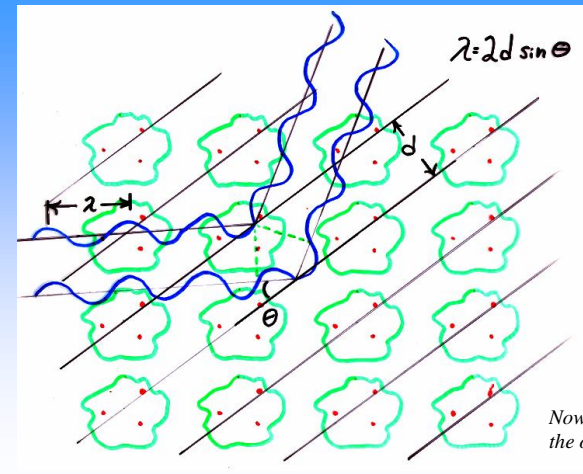


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Let's see if we can see how rays of "light" might interact with a crystal.



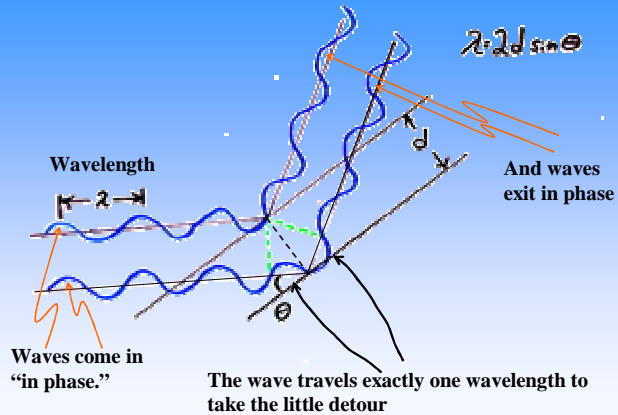
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Now get rid of the orchard...

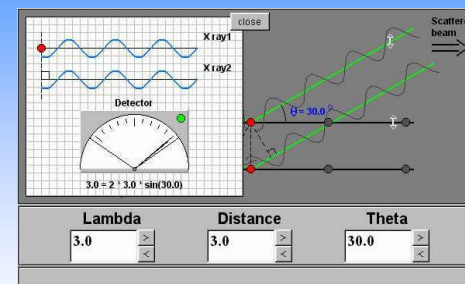
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Braggs' Law describes diffraction as reflection from planes



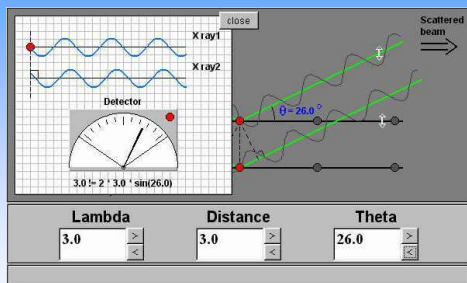
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Watch what happens as we go from maximum to minimum diffracting angle and back.

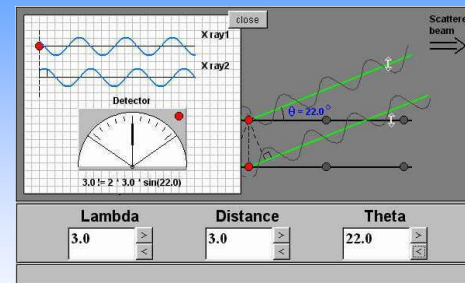


See: www.journeysunysb.edu/ProjectJava/Bragg/home.html

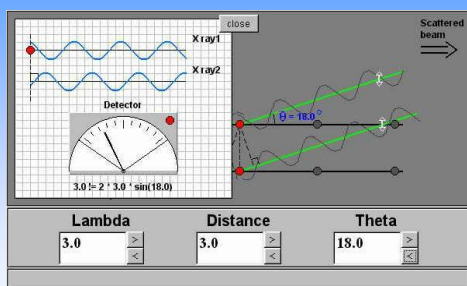
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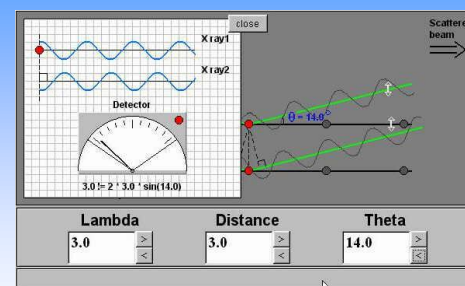
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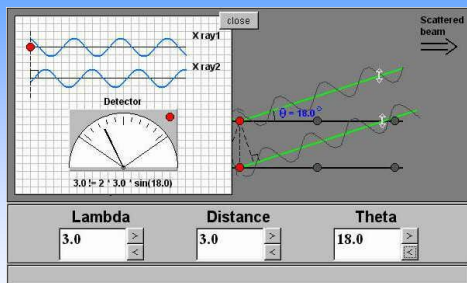
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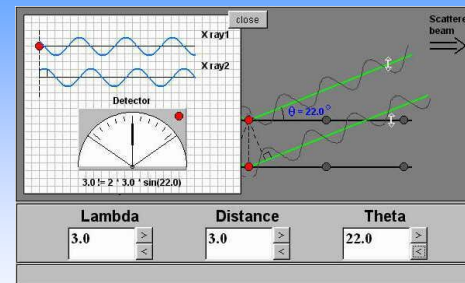
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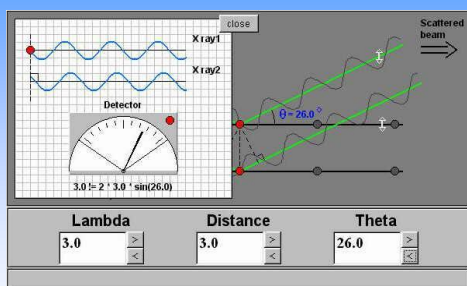
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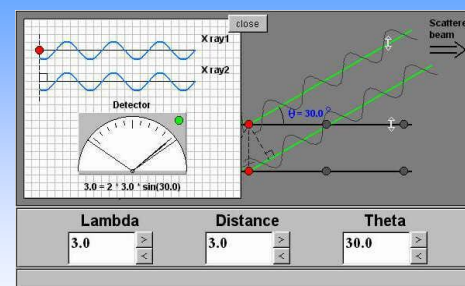
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Here's a two-dimensional example where we build up a crystal with a six-atom molecule and examine the diffraction pattern.

Each spot represents the intensity of reflection from one set of planes cutting through the crystal.

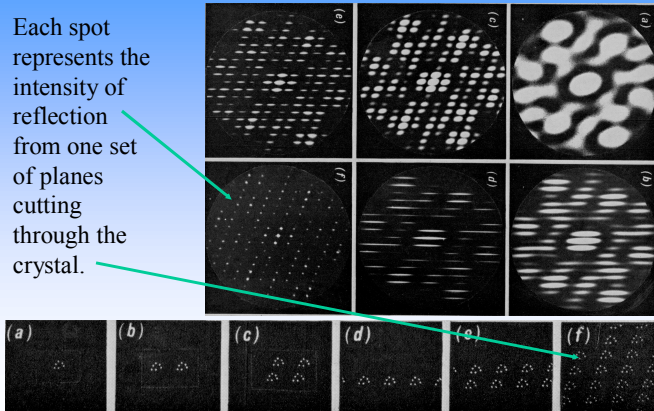
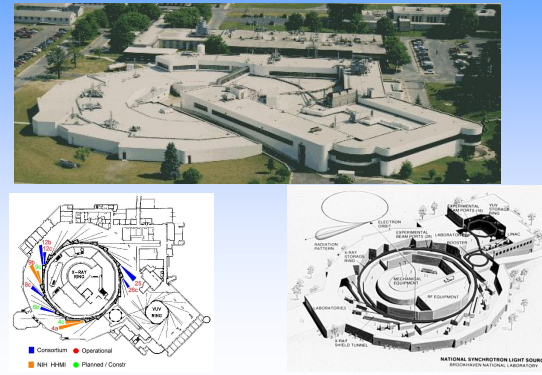


Plate 26 from Taylor and Lipson -- Optical Transforms

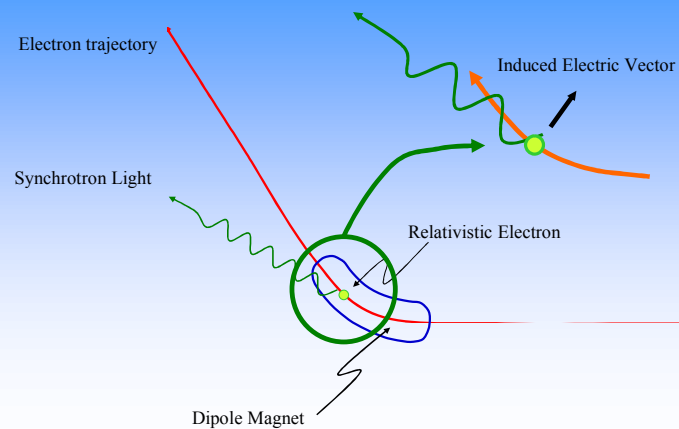
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Brookhaven Scientists use **X-Rays** from the **National Synchrotron Light Source** and lots of computing power to determine these structures



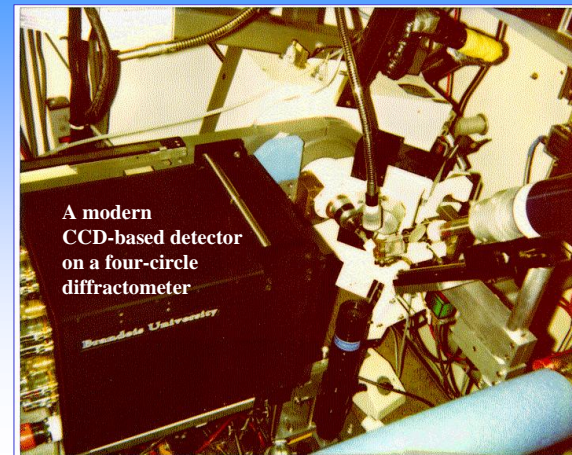
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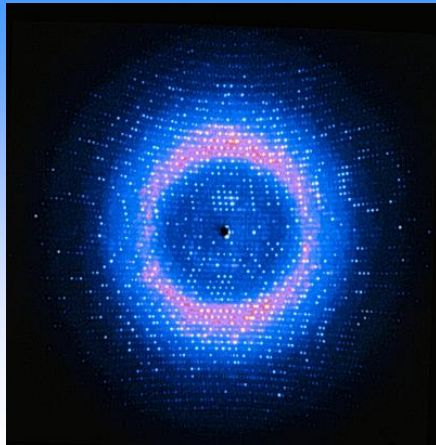
The Source of Synchrotron Radiation



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Complex apparatus allows us to measure diffraction patterns from protein crystals



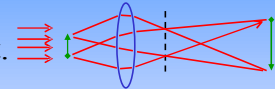


Simple rotation geometry produces a complicated pattern that requires good software to interpret. Modern CCD-based detectors with four-circle diffractometers record such patterns and measure every spot intensity.

An antique rotation photograph of B-Phyocerythrin -- real x-ray film. Brookhaven Biology

Q: How do we perform the second interference step in the functioning of the lens -- to **reconstruct the image** of the original object?

A: We will have to **calculate** it.



Q: How will we represent that object?

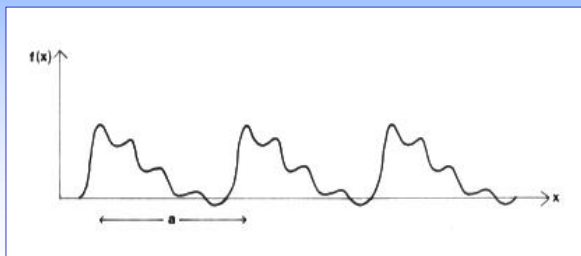
A: The **x-rays** are scattered from **electrons** in the atoms of the crystal.

Therefore: for us, the “**image**” is going to be a representation of the **electron density**.

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We use Fourier synthesis to calculate electron density. How does this work?

Can we produce a trial structure and see how waves can be summed to give this structure back?



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In the **Fourier Synthesis**, just a few waves suffice to give a reasonable approximation to the original pattern.

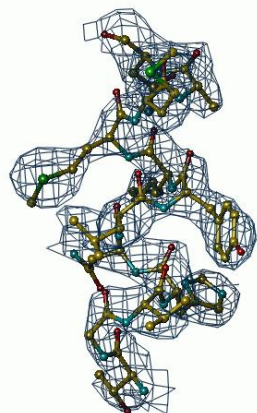
n	A_n	ϕ_n	f_n	$\sum_{n=1}^N f_n$
0	1.00	0		
1	1.21	0.6π		
2	0.46	0.8π		
3	0.32	0.9π		
4	0.26	0.8π		
5	0.29	1.3π		

The amplitudes are the square-root of the intensity of diffraction.

There are several ways to estimate the phase of diffraction.

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S-adenosyl homocysteine hydrolase



Turner *et al.*, Nat. Str. Bio 5 369-76 (1998)

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Typically, we represent regions of high electron density with “chickewire” cages like this.

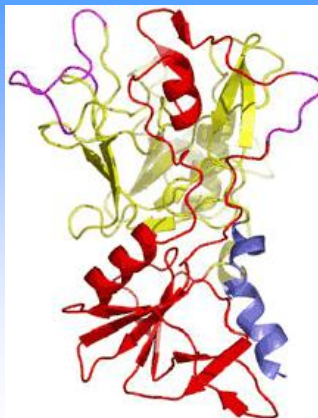
The molecular model can be built to fit inside.

Here are a couple more examples...

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Malaria is a serious world-wide health problem.

It infects approximately 400 million people worldwide, and kills about two million people each year — mostly children. This image shows the structure of an important protein that’s located on the outer surface of the parasite that causes **malaria**. It’s called **Apical Membrane Antigen-1**.



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Strategies for treatment of malaria

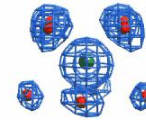
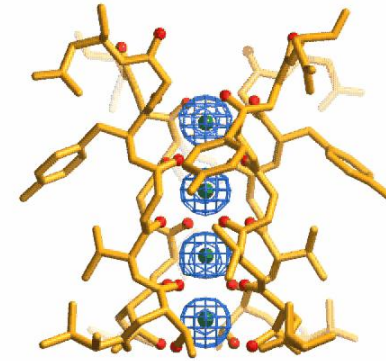
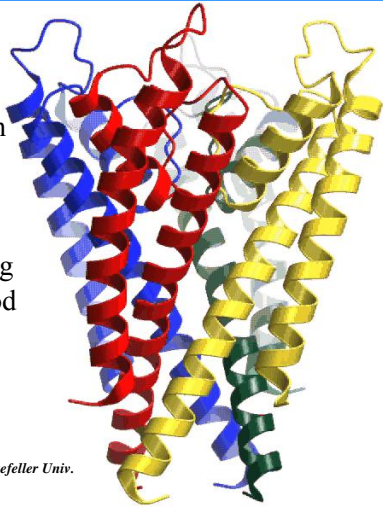
Brookhaven’s Mike Becker is part of the team that solved this structure. This molecule itself may be an effective part of a **vaccine**. In addition, features of the molecule suggest that it might be target for a drug that might be designed to bind to it.



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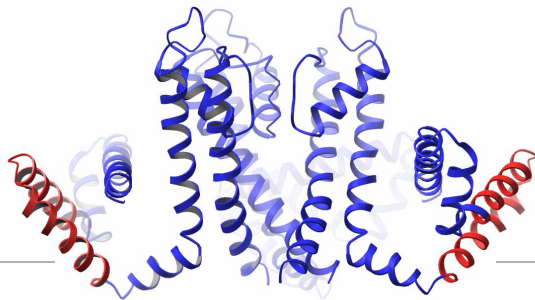
Some portion
of the work
performed in
the Nobel-
Prize-winning
studies of Rod
Mackinnon
was also
performed at
the NSLS.

Rod MacKinnon, Rockefeller Univ.



Rod MacKinnon, Rockefeller Univ.

Closed

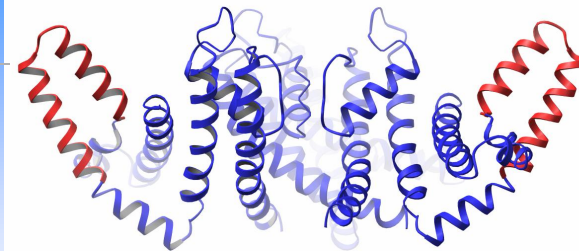


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Rod MacKinnon, Rockefeller Univ.

Opened



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Rod MacKinnon, Rockefeller Univ.

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Final Thoughts:

- We are in a golden age for biomedical research.
- Structural biology plays an important role by providing a tangible image of life processes.
- No single technique provides all the answers; we must collaborate.
- Synchrotron x-ray sources are a crucial component.
- We also depend on collaboration among National funding agencies.

